Journal of Chromatography, 344 (1985) 403—407
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2713

Note

Doxazosin determination by high-performance liquid chromatography using fluorescence detection

M.G. COWLISHAW* and J.R. SHARMAN

Toxicology Laboratory, Department of Clinical Biochemistry, Christchurch Hospital, Christchurch (New Zealand)

(First received March 5th, 1985; revised manuscript received May 14th, 1985)

Doxazosin is a quinazoline derivative, structurally related to prazosin and demonstrates similar clinical effects [1]. It is a vasodilator acting peripherally as an antagonist by selectively blocking post-synaptic α-adrenoreceptor sites [2, 3].

Prazosin has previously been used in the treatment of hypertension and associated cardiac failure, but it has two disadvantages: it requires multiple daily administration and there is a possible risk of postural hypotension. In contrast, doxazosin has a longer half-life and therefore a once daily administration may be adequate. Additionally, its slower onset of action may lessen the first-dose hypotension tendency seen with prazosin [2].

A method for determining doxazosin concentrations was required for a clinical trial to study the kinetics of the drug in renal failure [4].

Sensitive high-performance liquid chromatographic (HPLC) methods with fluorescent detection have been reported for doxazosin [5] and prazosin [6—10], and are stated to detect levels of 1 ng/ml and lower.

Initially attempts were made to use the method of Rubin et al. [5], but in our hands this was found to lack sensitivity, give poor reproducibility and to be time-consuming. The various steps in the method were investigated.

EXPERIMENTAL

Reagents and equipment

Methanol (HPLC grade), dichloromethane and diethyl ether were Baker Analysed Reagents (J.T. Baker, Phillipsburg, NJ, U.S.A.). Diethyl ether was

0378-4347/85/$03.30

routinely purified by removal of peroxides by washing with a 10% solution of sodium metabisulphite and distillation from glass.

Tetramethylammonium hydroxide (pentahydrate) and 1-pentane sulphonic acid sodium salt were purchased from Sigma (St. Louis, MO, U.S.A.).

The mobile phase consisted of 300 ml of a solution containing 0.01 mol/l aqueous pentane sulphonic acid and 0.02 mol/l tetramethylammonium hydroxide, buffered with glacial acetic acid to pH 3.4 plus 700 ml of 0.01 mol/l pentane sulphonic acid in methanol. This was pumped through the column at a flow-rate of 1.5 ml/min.

Chromatography was carried out on a 30 cm × 4.6 mm C_{18} μBondapak column, particle size 10 μm, at room temperature, using a Model M6000A pump, U6K injector and Model 420-E fluorescence detector (Waters Assoc., Milford, MA, U.S.A.). Excitation was achieved at 254 nm using an interference filter, and emitted light was measured using a 360-nm filter with a band width of 50 nm.

Standards

Pure doxazosin mesylate and prazosin hydrochloride were kindly provided by Pfizer (Sandwich, U.K.). Prazosin hydrochloride was used as the internal standard.

The doxazosin stock standard (50 μg/ml) was prepared by dissolving 5.0 mg of doxazosin in 100 ml of methanol. A 1-ml aliquot of the stock solution was diluted to 100 ml with water giving a concentration of 0.5 μg/ml (solution A). A final working standard of 10 ng/ml was prepared by diluting 0.5 ml of solution A to 25 ml with plasma and was used routinely with each batch of samples.

The internal standard stock solution (prazosin, 20 μg/ml) was prepared by dissolving 2.0 mg of prazosin in 100 ml of methanol. The working strength internal standard was made by diluting the stock solution with water to give a concentration of 10 ng/ml.

Extraction

All glassware was silanized by leaving overnight in an enclosed container saturated with dimethyldichlorosilane vapour.

A 1-ml volume of plasma or standard, 1 ml of internal standard, 200 μl of ammonia and 5 ml of dichloromethane—diethyl ether (1:2.5) were added to a 10-ml culture tube fitted with a Teflon®-lined screw cap. The tube was shaken for 2 min, the phases were separated by centrifugation for 10 min and the upper organic layer was transferred to a 10-ml conical centrifuge tube by means of a pasteur pipette. The solution was evaporated to dryness under a stream of nitrogen at 75°C. The residue was reconstituted with 20 μl of mobile phase and the tube vortexed to ensure solution. The tube was finally centrifuged for 1 min to collect all the liquid at the bottom. The total volume of 20 μl was used for a single injection onto the column.

The procedure described in this note was compared to the two-stage procedure of Rubin et al. [5]. Doxazosin and internal standard were extracted with diethyl ether, which was then cooled in dry ice—methanol to remove dissolved water. The dry diethyl ether was recovered and extracted with 20 μl of sulphuric acid (0.05 mol/l).

The steps of the two processes were investigated individually.

The first extraction step was examined. Doxazosin (10 ng/ml) and prazosin (10 ng/ml) were prepared separately in plasma. A 1-ml aliquot of each was extracted separately by our procedure. At the evaporation stage, 1 ml of methanolic prazosin was added to the doxazosin extract and similarly 1 ml of methanolic doxazosin to the prazosin extract. The peak heights were compared to those obtained following evaporation of methanolic standards.

The evaporation step was further studied by preparing doxazosin (10 ng/ml) and prazosin (10 ng/ml) separately in methanol. A 1-ml aliquot of each was added to one tube and evaporated to dryness under nitrogen. Chromatography solvent (20 μl) was added, the tubes were vortexed and the samples chromatographed. These samples were compared to aqueous samples made up to the concentration expected in the final solution and chromatographed.

At the final extraction stage Rubin et al. [5] used 0.05 mol/l sulphuric acid to recover doxazosin and internal standard from diethyl ether. This paper proposes the evaporation of a dichloromethane–diethyl ether extract and solution of the residue in 20 μl of chromatography solvent. This final step was examined using ten replicates of 40 ng/ml by each method.

RESULTS AND DISCUSSION

Silanization of glassware was found to be necessary to eliminate losses.

In our hands, dichloromethane–diethyl ether was found to recover 50% more internal standard than diethyl ether alone and this solvent was adopted.

The first extraction step, removing the drugs from alkaline plasma, gave recoveries of 97 and 95% for doxazosin and prazosin, respectively. Since no contaminants were found in the dichloromethane–diethyl ether phase after the initial extraction, the freezing step was omitted. Some alkali could conceivably be transferred at this stage and therefore ammonia was chosen as a base rather than sodium hydroxide to eliminate possible alkaline residues on evaporation. No significant differences in doxazosin recovery were found from ten samples processed using each alkali, indicating adequate control of pH with ammonia.

No losses of doxazosin or prazosin were found on evaporation of the dichloromethane–diethyl ether.

The final extraction step showed that extraction of doxazosin (40 ng/ml) and internal standard from diethyl ether into sulphuric acid had a variance of 20.7 while evaporation of dichloromethane–diethyl ether and solution in chromatography solvent gave a variance of 1.6 for the same concentration. The poor recovery and reproducibility of the acid extraction was possibly due to the small volume of acid. Larger volumes of acid were inappropriate as this reduced sensitivity due to dilution of the final extract.

A standard curve was prepared by extracting plasma solutions containing 2, 5, 10, 20 and 40 ng/ml doxazosin. This was found to be linear passing through zero and as a result the use of single-point standardisation was considered justified in each run of analyses. The calibration was linear over the range 0–40 ng/ml, with a slope of 0.99 ± 0.03, and a negligible y intercept (0.1 ± 0.6). The correlation coefficient was 0.995 and the standard error of estimate was 1.3. The detection limit of the assay was 0.5 ng/ml.

Reproducibility studies using three samples containing different concentrations of doxazosin gave the following coefficients of variation (C.V.); 2.2% at 20.0 ng/ml, 2.5% at 10 ng/ml and 4.6% at 2.0 ng/ml (Table I).

This method was used to measure doxazosin concentrations in 220 samples from a trial to study its kinetics in patients with renal failure [10]. Analyses were carried out in duplicate and the levels ranged from 0.5 to 19.5 ng/ml. The

TABLE I

PRECISION OF DOXAZOSIN ASSAY

Specimen	Concentration (mean ± S.D., n = 10) (ng/ml)	Coefficient of variation (%)
A	2.0 ± 0.07	4.6
B	10.2 ± 0.25	2.5
C	20.0 ± 0.45	2.2

TABLE II

DRUGS TESTED FOR POSSIBLE INTERFERENCE OF DOXAZOSIN ASSAY

Allopurinol	Netilmicin
Amphotericin	Nystatin
Antazoline	Paracetamol
Atropine	Pethidine
Beclomethasone	Prednisone
Bisacodyl	Promethazine
Colchicine	Quinine
Fluphenazine	Salbutamol
Frusemide	Triazolam
Flucloxacillin	Trimethoprim
Glyceryl trinitrate	Xylometazoline

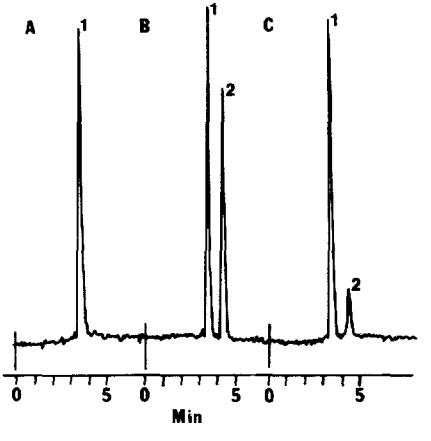

Fig. 1. Chromatograms obtained for the HPLC assay of doxazosin. (A) Plasma containing 10 ng/ml prazosin (internal standard); (B) plasma containing a doxazosin standard (10 ng/ml) and internal standard (10 ng/ml); (C) plasma sample of doxazosin (2 ng/ml) with internal standard (10 ng/ml). Peaks: 1 = prazosin; 2 = doxazosin.

patients were on a number of other medications and these drugs were tested for possible interference with the method. Solutions of the drugs were treated as samples, extracted by the above procedure and chromatographed. The drugs tested are listed in Table II.

There were no interfering peaks in blank plasma (Fig. 1A). Quinine was the only drug which chromatographed similarly to doxazosin and interfered with the analysis. Fig. 1B shows a plasma standard and Fig. 1C a plasma sample. Prazosin elutes at 3.4 min and doxazosin at 4.7 min.

The present method is a rapid, accurate HPLC procedure for the measurement of low levels of doxazosin, allowing approximately 50 samples per day to be analysed.

REFERENCES

1 J. Vincent, H. Elliott, P.A. Meredith and J.L. Reid, Brit. J. Clin. Pharmacol., 15 (1983) 719.
2 H.L. Elliott, P.A. Meredith, D.J. Sumner, K. Mclean and J.L. Reid, Brit. J. Clin. Pharmacol., 13 (1982) 699.
3 F. Ali Karamat, S.P. Kossen, P.B.M.W.M. Timmermans and P.A. van Zwieten, Brit. J. Pharmacol., 68 (1980) 113.
4 R. Carlson, R.R. Bailey, E.J. Begg, M.G. Cowlishaw and J.R. Sharman, submitted for publication.
5 P.C. Rubin, J. Brunton and P. Meredith, J. Chromatogr., 221 (1980) 193.
6 T.M. Twomey and D.C. Hobbs, J. Pharm. Sci., 67 (1978) 1468.
7 E.T. Lin, P.A. Baughman, Jr. and L.Z. Benet, J. Chromatogr., 183 (1980) 367.
8 L.G. Yee, P.C. Rubin and P. Meffin, J. Chromatogr., 172 (1979) 313.
9 P.A. Reece, J. Chromatogr., 221 (1980) 188.
10 J. Dokladalova, S.J. Coco, P.R. Lemke, G.T. Quercia and J.J. Korst, J. Chromatogr., 224 (1981) 33.